

# Phylogeography and Cryptic Species Diversity of *Paramesotriton caudopunctatus* Species Group (Salamandridae: *Paramesotriton*) in Guizhou, China

Tao LUO<sup>1\*</sup>, Huamei WEN<sup>1,2\*</sup>, Kai GAO<sup>3</sup>, Jun ZHOU<sup>1</sup> and Jiang ZHOU<sup>1\*</sup>

<sup>1</sup> School of Karst Science, Guizhou Normal University, Guiyang 550001, Guizhou, China

<sup>2</sup> School of Life Sciences, Central China Normal University, Wuhan 430000, Hubei, China

<sup>3</sup> Key Laboratory for Biodiversity and Ecological Engineering of Ministry of Education, College of Life Sciences, Beijing Normal University, Beijing 100875, China

**Abstract** The *Paramesotriton caudopunctatus* species group is mainly distributed in the karst mountain ecosystems of Guizhou, China. Although some species have been included in previous phylogenetic studies, the evolutionary relationships and divergence-time of members of this species group as a whole remain unexplored. In this study, we report the sequencing of one protein coding mitochondrial gene fragment (ND2) and one nuclear gene (POMC), and use a combination of phylogenetic analyses and coalescent simulations to explore the cryptic diversity and evolutionary history of the *P. caudopunctatus* species group. Phylogenetic relationships revealed that the *P. caudopunctatus* species group is composed of two major groups, i. e., East Clade and Western-South Clade. The divergence-time and ancestral area estimation suggested that the *P. caudopunctatus* species group likely originated in the Doupeng Mountains in Guizhou, China at 12.34 Ma (95% HPD: 8.30–14.73), and intraspecific divergence began at about 2.17 Ma (95% HPD: 1.39–2.97). This timing coincides with the orogenesis of the Miaoling Mountains during the Late Miocene to early Pleistocene. The delimitation of species in the *P. caudopunctatus* species group supports the existence of the currently identified species, and consensus was confirmed across methods for the existence of least to two cryptic species within what has been traditionally considered to be *P. caudopunctatus* species group. This study is of significance for understanding the species formation, dispersal, and diversity of the tailed amphibians in the karst mountains ecosystem of Guizhou and the role of the Miaoling Mountains as a geographical barrier to species dispersal.

**Keywords** *P. caudopunctatus* species group, phylogeography, cryptic diversity, biogeography, species delimitation, Guizhou

## 1. Introduction

The *Paramesotriton* Chang, 1935 of the Salamandridae are distributed in northern Vietnam and southwest-central, and southern China (Fei *et al.*, 2006; Frost, 2020). This genus currently contains 14 species (Frost, 2020), and noticeably, seven of these have been described in the past decade (Li *et al.*, 2008a, 2008b; Wu *et al.*, 2010; Gu *et al.*, 2012a; Wang *et al.*, 2013; Yuan *et al.*, 2014, 2016a). Except for *P. deloustali* (Bourret, 1934), all other species are endemic to China, and 14 of them are assigned into two species groups (Fei *et al.*, 2006; Yuan *et al.*, 2014): (1) the *P. caudopunctatus* species group, five species: *P. caudopunctatus* (Liu and Hu, 1973), *P. wulingensis* Wang, Tian, and Gu, 2013, *P. longliensis* Li, Tian, Gu, and Xiong, 2008, *P. zhiijinensis* Li, Tian, and Gu, 2008 and *P. maolanensis* Gu, Chen, Tian, Li, and Ran, 2012; (2) and the *P. chinensis* species group, nine species: *P. aurantius* Yuan, Wu, Zhou, and Che, 2016, *P. chinensis* (Gray, 1859), *P. deloustali* (Bourret, 1934), *P. fuzhongensis* Wen, 1989, *P. guangxiensis* (Huang, Tang, and Tang, 1983), *P. hongkongensis* (Myers and Leviton, 1962), *P. labiatus* (Unterstein, 1930), *P. qixilingensis* Yan, Zhao, Jiang, Hou, He, Murphy, and Che, 2014, and *P. yunwuensis* Wu, Jiang, and Hanken, 2010. The diversity of *Paramesotriton* spp. has been surveyed in several southern provinces of China, and several new species have been described in the provinces of Guizhou (Li *et al.*, 2008a, 2008b; Gu *et al.*, 2012a; Wang *et al.*, 2013), Guangxi (Wu *et al.*, 2009), Guangdong (Wu *et al.*, 2010), Hubei (Yuan *et al.*, 2016a), and Jiangxi (Yuan *et al.*, 2014). Given the taxonomic diversity of these salamanders, additional investigations are needed to develop effective plans for their conservation and management.

The *P. caudopunctatus* species group represents several taxa of mountains stream salamanders, narrowly distributed in

\* Both authors contributed equally to this work.

\* Corresponding author: Prof. Jiang ZHOU, from Guizhou Normal University, Guiyang, China, with his research focusing on zoology.  
E-mail: zhoujiang@ioz.ac.cn

Received: 5 March 2020 Accepted: 7 January 2021

medium to high elevation areas in the Guizhou Province, China (Fei *et al.*, 2016). Historically it was considered a single species (Fei *et al.*, 2006). Currently, *P. caudopunctatus* is listed as near threatened by the IUCN (<https://www.iucnredlist.org/species/59456/11945015>) and as vulnerable on the Red List of China's Vertebrates due to habitat degradation and overfishing, which has led to a decline in its population (Fei *et al.*, 2012; Jiang *et al.*, 2016). *P. longliensis*, *P. zhijinensis*, and *P. maolanensis* are listed as endangered due to their narrow area of distribution and limited population size (Fei *et al.*, 2012, 2016). Previous taxonomic studies have shown that the *P. caudopunctatus* species group is composed of several species, including *P. caudopunctatus*, *P. wulingensis*, *P. longliensis*, *P. zhijinensis*, and *P. maolanensis* (Yuan *et al.*, 2014). This suggests that the species diversity of this group may be severely underestimated. Consequently, a comprehensive assessment of the species number contextualized with their evolutionary history is necessary to identify the species conservation status. This is essential in order to develop an effective management plan and a better understanding of the biological history of the *P. caudopunctatus* species group.

Mountains ecosystems are characterized by high biodiversity, with evidence of species showing a wide range of evolutionary adaptations (Elsen *et al.*, 2015; Körner *et al.*, 2002; McCain *et al.*, 2011). They also serve as sanctuaries for many endemic and threatened species and thus play a major role in maintaining biodiversity (Favre *et al.*, 2016). Mountains ecosystems provide key ecological service functions and provide important natural resources that are utilized by local human populations (Grêt-Regamey *et al.*, 2012; Körner *et al.*, 2002). Thus, mountains species are currently at higher risk of extinction due to their limited distribution, unique environmental adaptability, and geographical isolation. They also are highly threatened in the context of climate change (Wang *et al.*, 2018; Yan *et al.*, 2018; Hu *et al.*, 2019).

In the case of salamanders, the mitochondrial NADH dehydrogenase subunit 2 gene (ND2) and the nuclear proopiomelanocortin protein gene (POMC) are commonly used molecular genetic markers in species identification and amphibian phylogeny (Vieites *et al.*, 2007; Wang *et al.*, 2018). These two genes can effectively identify evolutionarily significant units and management units in tailed amphibians (Stuart *et al.*, 2010; Gu *et al.*, 2012a; Wu *et al.*, 2010; Yuan *et al.*, 2014, 2016a).

Here, based on the most complete sampling (including two putative species) to date, we examine the phylogeny and biogeography of the *P. caudopunctatus* species group using both mitochondrial and nuclear data. Specifically, we aim to: (1) explore interspecific relationship within the *P. caudopunctatus* species group; (2) infer the center of origin and estimate dates for each divergence event; and (3) employ a series of species-

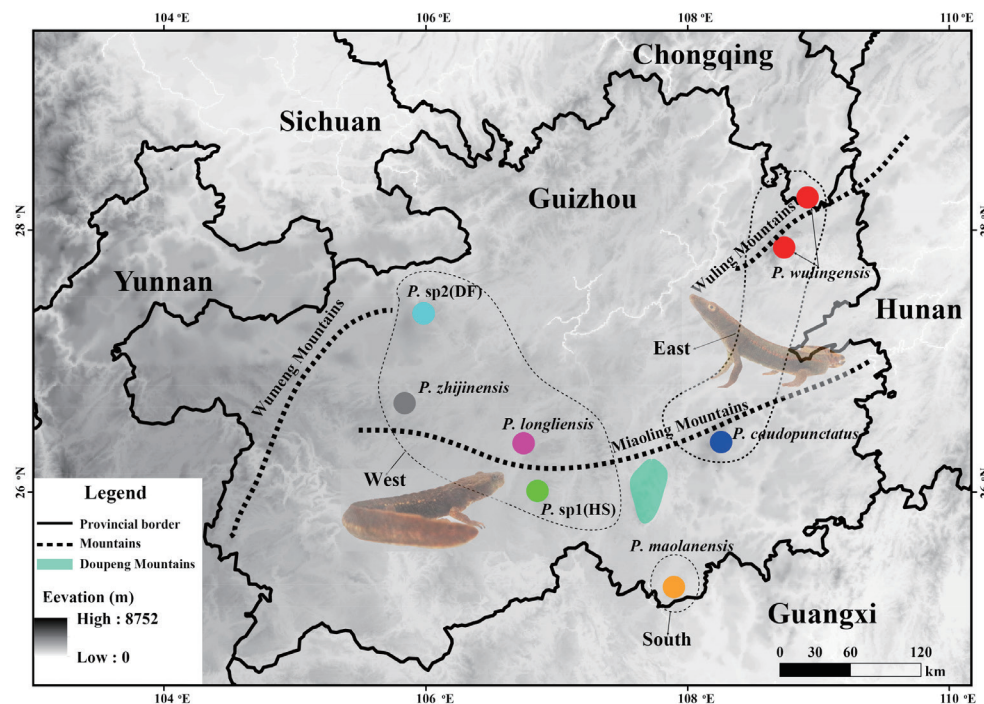
delimitation methods to clarify species boundaries and to identify candidate species among taxa in the *P. caudopunctatus* species group.

## 2. Materials and Methods

**2.1. Taxon sampling and molecular data collection** The *P. caudopunctatus* species group is mainly distributed in the Wuling Mountains, Miaoling Mountains and Wumeng Mountains in Guizhou. The molecular data from the vast majority of these areas have been stored in the National Center for Biotechnology Information (NCBI, USA). However, samples from the southern part of the Miaoling Mountains and the northern part of the Wumeng Mountains are still lacking. Therefore, 10 samples were collected from both regions for molecular analysis, they are: Huishui (HS, six individuals) and Dafang (DF, four individuals) (Figure 1). All muscle samples were attained from euthanized specimens and then preserved in 95% ethanol and stored at  $-40^{\circ}\text{C}$ . Additional information for these samples is presented in Table 1 and Figure 1. In addition, we downloaded 44 sequences from the NCBI database for molecular analysis and determined whether the sequences were from the same individual based on the specimen voucher number. Finally, 42 ND2 sequences and 35 POMC sequences were used in the molecular analyses.

**2.2. DNA extraction and sequencing** Total DNA was extracted using the standard phenol-chloroform extraction protocol (Sambrook *et al.*, 1989). All samples were sequenced for one mitochondrial gene and one nuclear gene, i.e. the partial NADH dehydrogenase subunit 2 gene (ND2) and the proopiomelanocortin gene (POMC). Primers used for ND2 were Ile3700L (5'-AGGRRYYACTTTGATARAGT-3') and COI5350H (5'-AGGGTGCCRATRTCYTTRTGRTT-3') following Zhang *et al.* (2008), and for POMC were POMC-F (5'-ACTGCAGGAAATAAGAGAGAAG-3') and POMC-R (5'-GAGTCATTAGAGGTTTTTGACT-3') from this study. Amplification was performed in 25- $\mu\text{L}$  reaction mixes using the following procedure: initial denaturation at  $95^{\circ}\text{C}$  for 5 min; followed by 35 cycles of denaturation at  $95^{\circ}\text{C}$  for 45 s, annealing at  $50^{\circ}\text{C}$  (for ND2) /  $48^{\circ}\text{C}$  (for POMC) for 1 min, and extension at  $72^{\circ}\text{C}$  for 70 s; and a final extension at  $72^{\circ}\text{C}$  for 10 min. The amplified PCR products were purified and sequenced in both directions using an ABI 3730 automated genetic analyzer. All sequences were deposited to GenBank. Some homologous DNA sequences of voucher specimens of related species were downloaded from GenBank and included in phylogenetic analyses (Table 1).

For the molecular analysis, a total of 75 nucleotide sequences of the *P. caudopunctatus* species group were used, including 40 ND2 sequences and 35 POMC sequences. In addition, two



**Figure 1** Map showing the distribution of the *P. caudopunctatus* species group in Guizhou Province, China used in this study. Colors in the pie-diagrams correspond to the time tree in Figure 2.

sequences were downloaded from GenBank as outgroups (i.e. *P. deloustali* and *P. hongkongensis*). Detailed information of these materials is shown in Table 1.

**2.3. Phylogenetic analysis** The resulting nucleotide sequences were first assembled and edited using DNASTAR LASERGENE 7.1. Sequences from ND2 and POMC genes were aligned using MUSCLE (Edgar, 2004) in MEGA 7.0 (Kumar *et al.*, 2016). In cases for which only mitochondrial data or nuclear loci were available for a species, we treated nuclear or mitochondrial partitions for these individuals as missing data. Based on the dataset using ND2+POMC, phylogenetic analyses were conducted using the maximum likelihood (ML) and Bayesian inference (BI) methods, as implemented in IQ-TREE 1.6.5 (Nguyen *et al.*, 2014) and MRBAYES 3.2 (Ronquist *et al.*, 2012), respectively. Prior to analyses, the best-fitting substitution models and partitioning schemes were selected in PARTITIONFINDER 2.1.1 using the Bayesian information criterion (Lanfear *et al.*, 2012). BI analyses were conducted with  $2 \times 10^7$  generations and sampled every 1,000 generations. Convergence was assessed in TRACER 1.7.1 (Rambaut and Drummond, 2007) based on the average standard deviation of split frequencies ( $< 0.01$ ) and ESS values ( $> 200$ ). We used the remaining samples to generate the consensus tree after omitting the first 25% trees as burn-in. ML analyses were conducted in IQ-TREE using ultrafast bootstrapping (Hoang *et al.*, 2018)

and run until these converged at a correlation coefficient of  $\geq 0.99$ . Nodes in the trees were considered well supported when Bayesian posterior probabilities (BPP) were  $\geq 0.95$  and ML ultrafast bootstrap values (UFB) was  $\geq 95\%$ . Pairwise distance ( $p$ -distances, 1 000 replicates) between lineages was calculated in MEGA 7.0 (Kumar *et al.*, 2016).

**2.4. Divergence time estimation and ancestral area reconstruction** In this study, our divergence time estimates were based solely on mitochondrial and nuclear genetic data. To understand the divergence times of the *P. caudopunctatus* species group, we used a relaxed molecular clock assumption in BEAST 1.8.2 (Drummond *et al.*, 2012). No reliable ranid fossil record is presently available to provide proper calibration within the *P. caudopunctatus* species group. Therefore, two divergence time calibration points were included in this study following the recommendations of Zhang *et al.* (2008): (1) the calculated age of 9.9–16.5 Ma for the split between *P. caudopunctatus* and *P. deloustali* + *P. hongkongensis* (normally distributed calibration density; mean = 13.2 Ma); (2) the calculated age of 4.4–11.8 Ma for the split between *P. deloustali* and *P. hongkongensis* (normally distributed calibration density; mean = 7.5 Ma). Divergence times were estimated using the optimal partitioning strategy as determined by PARTITIONFINDER and normal prior distributions. BEAST analyses were run for  $1 \times 10^7$  generations, with samples taken every 5,000 generations under a relaxed



**Table 1** Specimens included in the molecular phylogenetic analysis. An asterisk indicates newly obtained nucleotide sequence data from this study. GZNU: Guizhou Normal University, China; KIZ: Kunming Institute of Zoology, Chinese Academy of Sciences, China.

ID	Species	Voucher number	Locality	ND2	POMC
1	<i>Paramesotriton longliensis</i>	GZNU 20070421001	Longli, Guizhou, China	FJ169608	JQ680392
2	<i>Paramesotriton longliensis</i>	KIZ-GZH 081025	Longli, Guizhou, China	GU980576	–
3	<i>Paramesotriton longliensis</i>	GZNU 20070421004	Longli, Guizhou, China	JF438975	MW147293
4	<i>Paramesotriton longliensis</i>	GZNU 20070421003	Longli, Guizhou, China	JF438974	MW147292
5	<i>Paramesotriton longliensis</i>	GZNU 20070421002	Longli, Guizhou, China	JF438973	JQ680393
6	<i>Paramesotriton longliensis</i>	GZNU 2018061813	Huishui, Guizhou, China	MW147285	MW147297
7	<i>Paramesotriton longliensis</i>	GZNU 2018061814	Huishui, Guizhou, China	MW147283	MW147302
8	<i>Paramesotriton longliensis</i>	GZNU 20180618016	Huishui, Guizhou, China	MW147286	MW147296
9	<i>Paramesotriton longliensis</i>	GZNU 20180618017	Huishui, Guizhou, China	MW147284	MW147303
10	<i>Paramesotriton maolanensis</i>	GZNU 2006030003	Libo, Guizhou, China	FJ169607	JQ680398
11	<i>Paramesotriton maolanensis</i>	GZNU 2006030006	Libo, Guizhou, China	JF438972	–
12	<i>Paramesotriton maolanensis</i>	GZNU 2006030004	Libo, Guizhou, China	JF438993	JQ680399
13	<i>Paramesotriton maolanensis</i>	GZNU 2006030005	Libo, Guizhou, China	JF438994	JQ680400
14	<i>Paramesotriton</i> sp2(DF)	GZNU 20180709024	Dafang, Guizhou, China	MT811029	MT811033
15	<i>Paramesotriton</i> sp2(DF)	GZNU 2018070904	Dafang, Guizhou, China	MT811030	MT811034
16	<i>Paramesotriton</i> sp2(DF)	GZNU 2018070905	Dafang, Guizhou, China	MT811031	MT811035
17	<i>Paramesotriton</i> sp2(DF)	GZNU 2018070903	Dafang, Guizhou, China	MT811028	MT811032
18	<i>Paramesotriton</i> sp1(HS)	GZNU 2018061815	Huishui, Guizhou, China	MT811026	MT811036
19	<i>Paramesotriton</i> sp1(HS)	GZNU 2018061814	Huishui, Guizhou, China	MT811027	MT811037
20	<i>Paramesotriton zhijinensis</i>	GZNU 20070415002	Zhijin, Guizhou, China	JF438976	JQ680396
21	<i>Paramesotriton zhijinensis</i>	GZNU 20070415003	Zhijin, Guizhou, China	JF438977	MW147294
22	<i>Paramesotriton zhijinensis</i>	GZNU 20070415004	Zhijin, Guizhou, China	JF438978	MW147295
23	<i>Paramesotriton zhijinensis</i>	GZNU 20070415001	Zhijin, Guizhou, China	FJ169609	JQ680397
24	<i>Paramesotriton zhijinensis</i>	KIZ-GZH 081026	Zhijin, Guizhou, China	GU980575	–
25	<i>Paramesotriton wulingensis</i>	GZNU 07072001	Jiangkou, Guizhou, China	FJ938040	MW147291
26	<i>Paramesotriton wulingensis</i>	GZNU 2007071002	Jiangkou, Guizhou, China	JF438987	MW147290
27	<i>Paramesotriton wulingensis</i>	GZNU 2007071004	Jiangkou, Guizhou, China	JF438989	JQ680385
28	<i>Paramesotriton wulingensis</i>	GZNU 08072603	Youyang, Chongqing, China	JF438991	JQ680388
29	<i>Paramesotriton wulingensis</i>	GZNU 08072602	Youyang, Chongqing, China	JF438990	JQ680387
30	<i>Paramesotriton wulingensis</i>	GZNU 2007071003	Youyang, Chongqing, China	JF438988	MW147289
31	<i>Paramesotriton wulingensis</i>	GZNU 08072604	Youyang, Chongqing, China	JF438992	MW147301
32	<i>Paramesotriton wulingensis</i>	KIZ 21898	Fanjingshan, Guizhou, China	KJ650055	–
33	<i>Paramesotriton wulingensis</i>	KIZ 21899	Fanjingshan, Guizhou, China	KJ650056	–
34	<i>Paramesotriton caudopunctatus</i>	GZNU 2007071001	Leishan, Guizhou, China	FJ169606	JQ680386
35	<i>Paramesotriton caudopunctatus</i>	GZNU 200904251	Leishan, Guizhou, China	MW147288	JQ680390
36	<i>Paramesotriton caudopunctatus</i>	GZNU 200904252	Leishan, Guizhou, China	MW147287	JQ680389
37	<i>Paramesotriton caudopunctatus</i>	GZNU 2007072005	Leishan, Guizhou, China	JF438986	JQ680391
38	<i>Paramesotriton caudopunctatus</i>	GZNU 2009042501	Leishan, Guizhou, China	JF438983	MW147299
39	<i>Paramesotriton caudopunctatus</i>	GZNU 20050727001	Leishan, Guizhou, China	JF438985	MW147298
40	<i>Paramesotriton caudopunctatus</i>	GZNU 20050727002	Leishan, Guizhou, China	JF438984	MW147300
41	<i>Paramesotriton deloustali</i>	MVZ 223628	Tam Dao, Vinh Phu, Vietnam	EU880327	–
42	<i>Paramesotriton hongkongensis</i>	Not mention	Shenzhen, Guangdong, China	AY458597	–

molecular clock and a Yule tree prior. Stationarity was assessed using TRACER 1.7.1, with ESS values >200 taken as evidence of convergence. Maximum clade credibility (MCC) trees were obtained using TREEANNOTATOR 2.4.1 by applying a burn-in of 25%.

The ancestral range was reconstructed using Dispersal-Vicariance Analysis (S-DIVA) implemented in the program RASP 3.2 (Yu *et al.*, 2014). We reconstructed an mtDNA and nDNA genes tree using one representative of each taxon based on the same sets as BI analyses, and a total of 1000 post-burn-in trees were used. Each sample was coded to represent the distribution of the species, and five distributional areas were used: Leigong Mountains (LM), Wuling Mountains (WL), Southern Guizhou (SG), and west of Miaoling Mountains (WM). The maximum number of areas was kept as two.

**2.5. Species delimitation** We used SPLITSTREE 4.16.1 (Huson and Bryant, 2006) to construct a phylogenetic network based on uncorrected *p*-distances with heterozygous ambiguities averaged and normalized, using the neighbor-net ordinary least squares variance and equal angle algorithm and 1000 bootstrap replicates to assess branch support. We used the Bayes factor (BF) approach (Grummer *et al.*, 2014) to estimate the best fitting model to our dataset between alternative models (M1: 7 species; M2: 6 species; M3: 5 species; M4: 4 species; M5: 3 species; M6: 2 species; M7: 1 species), defined by the estimates of population structure identified using the above phylogenetic tree. Marginal model likelihood used in Bayesian model comparisons (Bergsten *et al.*, 2013) was calculated via both path-sampling (PS; Baele *et al.*, 2012) and stepping-stone (SS; Xie *et al.*, 2011) methods in BEAST 1.8.2 (Drummond *et al.*, 2012). Substitution models for mtDNA and nDNA were determined using PARTITIONFINDER 2.1.1. The Marginal likelihood estimate (MLE) of each model was estimated and the BF between pairs of modes was calculated as  $BF = 2 \times (\text{best MLE model} - \text{worst MLE model})$ , with values for BF between 0 and 1 indicating very weak support for model 1 over 2, values between 1 and 3 indicating some support, albeit little, for model 1, values between 3 and 5 indicating strong support for model 1, and values >5 indicating decisive support for model 1 (Kass and Raftery, 1995). Two independent runs for each model were performed in BEAST 1.8.2 to assess convergence of the MCMC runs. \*BEAST was run each time for  $1 \times 10^7$  generations of the MCMC algorithm sampling every 1 000 generations and discarding the first 25% of the iterations as “burn-in”. The clock model parameter settings were a strict clock normal model of lineage variation, a Yule Process prior for the branching rates, and an HKY model of sequence evolution. For MLE analysis, the applied parameters were as follows:  $1 \times 10^7$  generations, sampling every 1 000 generations and default settings for the other parameters. The results for different runs were combined

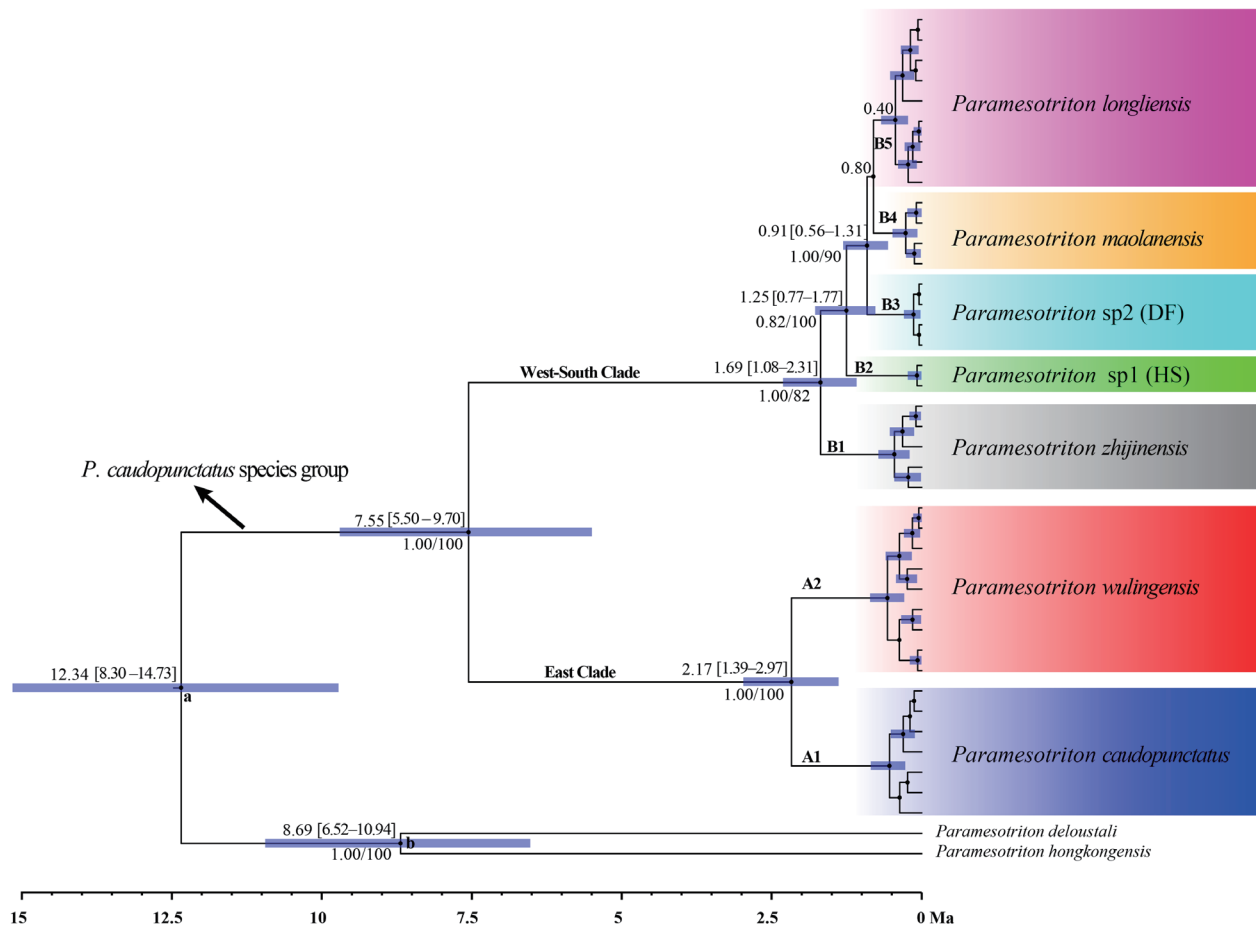
using LogCombiner. Based on the MLE results, the species tree of *P. caudopunctatus* species group was determined. Convergence of all model parameters was assessed by examining the trace plots and histograms in TRACER 1.7.1.

In addition to the Bayesian methods tested, we also applied three tree-based species-delimitation methods. They are the single-threshold General Mixed Yule Coalescent model (sGMYC; Fujisawa and Barraclough, 2013), the multiple threshold GMYC model (mGMYC; Monaghan *et al.*, 2009), and the Bayesian implementation of the Poisson Tree Processes model (bPTP; Zhang *et al.*, 2013). All three analyses were calculated using an online server (<http://species.h-its.org/>). BEAST's ultrametric tree with two outgroups (*P. deloustali*, *P. hongkongensis*) was used for the sGMYC, mGMYC and bPTP models with default parameter settings in the server. The parameters of these three analyses were set as follows: 500,000 generations, a thinning of 500 and burn-in of 10%. Convergence of models were assessed by visualizing plots of the MCMC iteration vs. the Log likelihood results. Lastly, we used the computationally efficient distance-based species-delimitation method ABGD (Puillandre *et al.*, 2012), which can quantify the barcode gap location that separates intra- from interspecific distances. During the calculation, default settings were used for the prior range of maximum intraspecific divergence (0.001, 0.1) and minimum slope increase (X) of 1.5 (default) and 1.0. K80 corrected distances were used to compare species delimitation results.

### 3. Results

**3.1. Sequence information** The aligned ND2 and POMC genes from the *P. caudopunctatus* species group and outgroups consisted of 1 864 bp nucleotide positions after trimming. This included 1 404 bp from ND2 and 460 bp from POMC. The trimmed data were used for genealogical reconstructions, including 1 570 constant and 294 variable sites. The partitions and best-fit evolutionary models for the data were as follows: HKY+I for the first and the second positions of ND2, HKY+G for the third position of the ND2, F81+I for POMC. All novel sequences generated have been deposited in GenBank (Table 1).

**3.2. Phylogenetic analysis** The ML and BI analyses resulted in essentially identical topologies and were integrated in Figure 2. In the phylogenetic tree, except for the outer-groups, the monophyly of the *P. caudopunctatus* species group was strongly supported (BPP = 1.00, UFB = 100%). The inner-group contained the East Clade, which consisted of *P. caudopunctatus* and *P. wulingensis* regions in the east of Guizhou, and West-South Clade, which was composed of the *P. longliensis*, *P. sp1* (HS), *P. zhijinensis*, *P. sp2* (DF), and *P. maolanensis* regions in the west and south of Guizhou (BPP = 1.00, UFB = 100%). East Clade



**Figure 2** Mitochondrial and nuclear gene sequences phylogeny of the *P. caudopunctatus* species group. The values below the node indicate Bayesian posterior probabilities and ML ultrafast bootstrap support (shown as a percentage). Letters (a and b) indicate the calibration points. The blue line at each node correspond to the 95% highest posterior density of the age of that node. The bottom axis is in millions of years.

consisted of two lineages, i.e. lineages A1 and A2. Lineage A1 was composed of seven samples from the Leigong Mountains, and lineage A2 was composed of nine samples from two populations (from Jiangkou and Youyang) in the Wuling Mountains area. The West-South Clade consisted of five lineages. Lineages B1 and B3 were composed of five samples from Zhijin County in the eastern part of the Wumeng Mountains and four samples from Dafang County; lineages B2 and B4 were composed of two samples from Huishui County, located south of the Miaoling Mountains and four samples from Libo County. Lineage B5 was composed of nine samples from the southern part of the Miaoling Mountains (from Longli and Huishui). Overall, this dataset yielded well-supported phylogenetic trees (BI and ML; Figure 2), reflecting the same topological structure previously identified for the *P. caudopunctatus* species group (Gu et al., 2012b; Yuan et al., 2014).

### 3.3. Divergence dating and ancestral-area estimation

The

two BEAST runs converged after  $1 \times 10^7$  generations, yielding ESS values  $> 200$  for all parameters, and producing highly congruent trees and dates. Figure 2 reveals the divergence times between different species of the *P. caudopunctatus* species group. Results indicate with weakly support (Probability = 0.50) that the *P. caudopunctatus* species group likely originated in the middle range of the Miaoling Mountains (current location of the Doupeng Mountains, including Majiang and Guiding) during the late Miocene (12.34 Ma; 95% HPD: 8.30–14.73). The divergence time of West-South Clade and Eastern Clade of the *P. caudopunctatus* species group was estimated to 7.55 Ma (95% HPD = 5.50–9.70). The divergence time of *P. caudopunctatus* and *P. wulingensis* was estimated to be 2.17 Ma (95% HPD = 1.39–2.97), and that of *P. zhijinensis* and (HS, DF) (*P. maolanensis*, *P. longliensis*) was 1.69 Ma (95% HPD = 1.08–2.31).

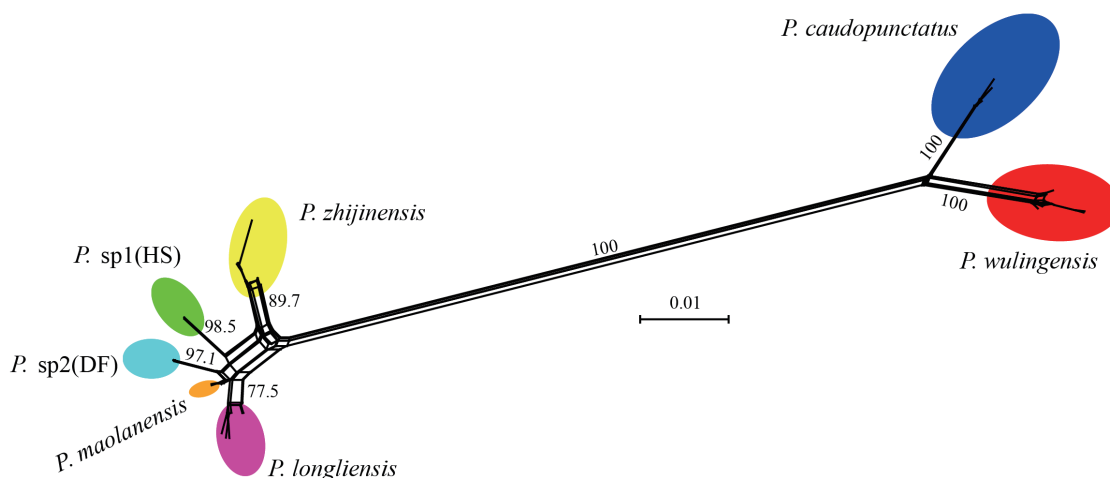
**3.4. Species delimitation and species tree** The phylogenetic network of the *P. caudopunctatus* species group contained

the same groupings observed use phylogenetic methods (Figure 3). For the Bayes-factor species delimitation, we compared the starting delimitation of seven species derived from mitochondrial and nuclear gene sequences reciprocal monophyly using seven alternative models (Table 2) that assumed fewer species. PS and SS ranked the seven delimitation models identically and with highly similar marginal likelihood estimates. The starting delimitation received the highest likelihood value, and the five-species model yielded the lowest value. Between the starting and alternative delimitations, BF ranged from 12.84 to 189.13 by PS, and from 12.70 to 190.06 by SS, decisively supporting the recognition of seven species. Because the seven-species delimitation constituted the optimal model, we used the corresponding \*BEAST species-tree for further analyses (Figure 4). Noteworthy is the fact that nodal support was low for basal relationships.

Four out of five of the species-delimitation methods consistently identified seven species, while the bPTP identified more than six (~6.55). The areas of Leishan, Jiangkou, Youyang, Zhijin, Dafang, Libo, and Longli consistently presented one species per area. Finally, in terms of genetic differences, average pairwise sequence divergence varied markedly among these species, from 0.61% (*P. maolanensis* vs *P. longliensis*) to 9.74% (*P. caudopunctatus* vs *P. zhijinensis*) (Table 3).

## 4. Discussion

**4.1. Phylogenetic relationships of the *P. caudopunctatus* species group** In this study, we conducted a large-sample comprehensive phylogenetic analysis of the *P. caudopunctatus* species group of the genus *Paramesotriton*, including 10 newly acquired taxonomic samples. Based on the mitochondrial gene

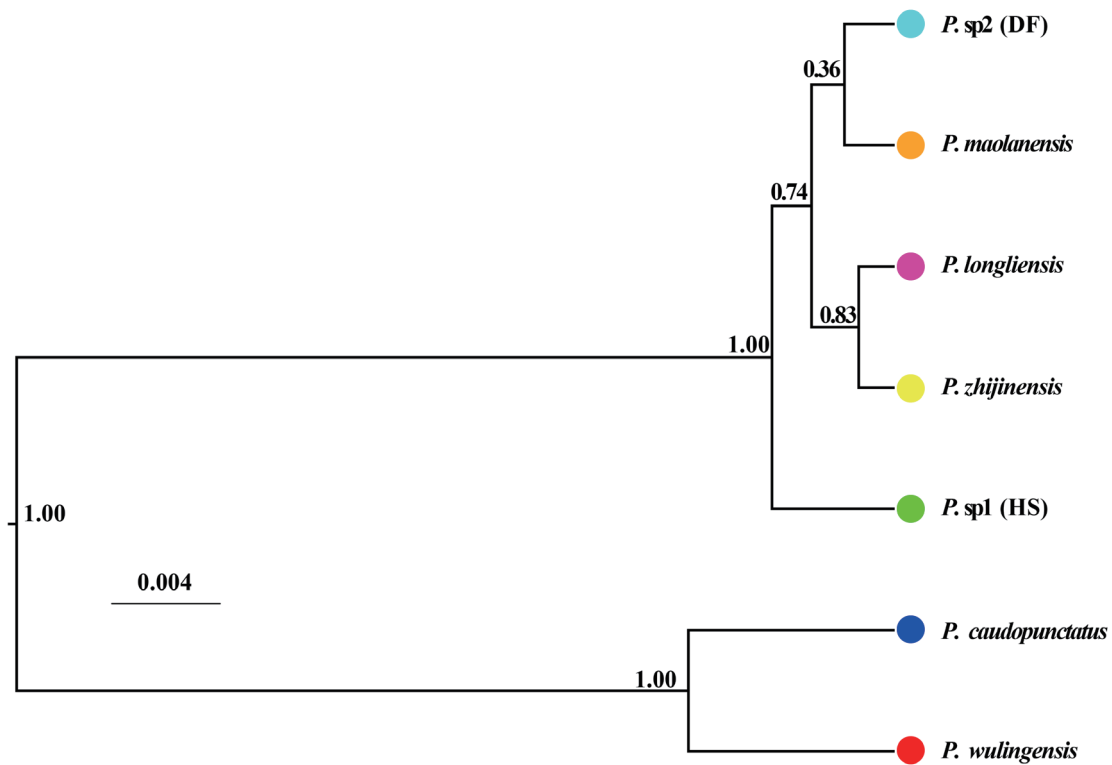


**Figure 3** Network constructed from the ND2 sequence of the *P. caudopunctatus* species group samples based on uncorrected *p*-distances using SPLITSTREE. The value at each node indicates bootstrap support (only values above 70% are shown).

**Table 2** Species delimitation results of the *P. caudopunctatus* species group based on the BF method. “MLE” represents “Marginal likelihood estimate”; “BF” represents “Bayes factor”; “PS” represents path-sampling method; “SS” represents the stepping-stone method.

Model	Species	Species composition	MLE Path Sampling{PS}	MLE Stepping Stone{SS}	BF	
					PS	SS
M1	7	PC, PW, PZ, HS, DF, PM, PL	−3973.10	−3973.14	N/A	N/A
M2	6	PC, PW, PZ, HS, DF, {PM, PL}	−3979.52	−3979.49	12.84	12.70
M3	5	PC, PW, PZ, HS, {DF, PM, PL}	−3984.14	−3983.91	22.07	21.54
M4	4	PC, PW, PZ, {HS, DF, PM, PL}	−3987.84	−3987.87	29.48	29.47
M5	3	PC, PW, {PZ, HS, DF, PM, PL}	−3999.20	−3999.25	52.21	52.22
M6	2	{PC, PW}, {PZ, HS, DF, PM, PL}	−4008.19	−4008.05	70.17	69.82
M7	1	{PC, PW, PZ, HS, DF, PM, PL}	−4067.67	−4068.17	189.13	190.06

Each model represents a possible relationship for *P. caudopunctatus* species group with varying number of species. Abbreviation as: *P. caudopunctatus*: PC; *P. wulingensis*: PW; *P. zhijinensis*: PZ; *P. maolanensis*: PM; *P. longliensis*: PL; *P. sp1*: HS; *P. sp2*: DF.



**Figure 4** Species tree inferred from the seven-species delimited by BEAST. The values on nodes are Bayesian posterior probabilities.

**Table 3** Mean *p*-distance ND2 gene among the *P. caudopunctatus* species group used in this study.

ID	Species	1	2	3	4	5	6
1	<i>P. caudopunctatus</i>						
2	<i>P. wulingensis</i>	2.72					
3	<i>P. longliensis</i>	9.78	9.69				
4	<i>P. maolanensis</i>	9.59	9.50	0.61			
5	<i>P. zhijinensis</i>	9.74	9.65	1.82	1.42		
6	<i>P. sp1</i> (HS)	9.48	9.39	1.35	0.95	1.74	
7	<i>P. sp2</i> (DF)	9.48	9.39	1.03	0.63	1.84	1.38

sequence ND2 and the nuclear gene POMC, two major clades (the East and West-South Clades) were consistently identified for the *P. caudopunctatus* species group in Guizhou Province, China. This topology is similar to that of previous studies (Gu et al., 2012b; Yuan et al., 2014). The genus *Paramesotriton* is divided into three subgenera by Fei et al. (2016), i.e. *Paramesotriton*, *Allomesotriton*, and *Karstotriton*. The subgenus *Allomesotriton* includes *P. caudopunctatus* and *P. wulingensis*; and the subgenus *Karstotriton* includes *P. longliensis*, *P. zhijinensis*, and *P. maolanensis*. Among the two subgenera previously proposed based on morphological characters, the subgenus *Allomesotriton* in the East Clade and subgenus *Karstotriton* in the West-south Clade

were all identified as monophyletic. At present, the phylogenetic relationships between species within the subgenus *Karstotriton* have not been well resolved.

#### 4.2. Biogeography of the *P. caudopunctatus* species group

Although biogeographic studies on Asian amphibians have greatly increased in the last decade (Che et al., 2010; Yan et al., 2013; Chen et al., 2017; Wang et al., 2018; Yuan et al., 2018; Jiang et al., 2019; Wu et al., 2020), little attention has been paid to salamanders. The *P. caudopunctatus* species group appears to have had a relatively short history in Southwest China. Dating analyses indicate that *P. caudopunctatus* species group originated about 12.34 Ma, and that the sequential speciation



of each species took place during a period of 0.80–7.60 Ma (Figure 2). This timing coincides well with the orogenesis of the Miaoling Mountains (7–15 Ma) during the Late Miocene to early Pleistocene (Zhou *et al.*, 1993). Mountain building may have triggered changes in local climate, the development of complex terrain (including the uplifting of mountains and the formation of rivers) and the formation of heterogeneous environments (Shi *et al.*, 2006). The origin of the *P. caudopunctatus* species group is spatially and temporally consistent with the time of divergence estimated for the common distribution of other biological taxa (e.g., *Quasipaa boulengeri*, Yan *et al.*, 2013; *Scaptonyx fusicaudus*, He *et al.*, 2019) in the region, and the dates of speciation are concordant with some species of frogs (Wu *et al.*, 2020; Chen *et al.*, 2017; Zhou *et al.*, 2017), and snakes (Guo *et al.*, 2020). All interspecific divergence events date to less than 3.0 Ma, which corresponds with the rapid uplifting and westward movement of the Miaoling Mountains at approximately 3.40 Ma (Zhou *et al.*, 1993). This is related to the rapid uplifting of the Tibetan plateau during this period (Sun, 1997).

The Western part of Guizhou has attracted widespread attention from biologists as a center of origin and a corridor for their dispersal for several taxa (Yan *et al.*, 2013; Yuan *et al.*, 2016b; Yao *et al.*, 2018). For the *P. caudopunctatus* species group, the current geographic distribution and species tree distribution are consistent with the “dispersal-vicariance-dispersal” model. After the initial split, the common ancestor of the species group expanded its distribution into the central part of the ancient Miaoling Mountains and West of Guizhou. This resulted in geographical isolation, which in turn led to the formation of East and West Clades. Continuous uplifting of the Qinghai-Tibetan Plateau led to habitat heterogeneity and ecological divergence, thus resulting in vicariance, driving speciation. Subsequent vicariance also best explains the narrow distribution of these species. Within the Eastern Clade, isolation between *P. caudopunctatus* and *P. wulingensis* species occurred early in the Pleistocene with the breakup of the Miao Mountains and the Wuling Mountains (Zhou *et al.*, 1993). Within West-South Clade, the phylogenetic branching pattern also suggests a general West-to-South and West-to-North dispersal pattern, because species endemic to the western most part of Miaoling Mountains represent basal clades (Figure 1). This unique pattern of dispersion may be related to the numerous rivers in the area.

**4.3. Species validity and cryptic species diversity** Since the description of *P. longliensis* and *P. zhijinensis* in Guizhou, China, it has been suggested that the limited genetic divergence between *P. longliensis* and *P. zhijinensis* indicates that the two names may describe the same (single) species (Wu *et al.*, 2010). In this study, we amplified and sequenced the mitochondrial and nuclear gene sequences of both species from specimens deposited in our laboratory from the type locality. In addition,

sequence information was collected from relevant literature. Two lines of evidence provide support for the validity of *P. longliensis* and *P. zhijinensis* as separate species. First, the BFD, GMYC single, GMYC multiple, bPTP, and ABGD analyses support the recognition of *P. longliensis* and *P. zhijinensis*. Second, In the species-tree (Figure 4), *P. longliensis* and *P. zhijinensis* were clearly distinguished as monophyletic, although the posterior probability between them was relatively low (BPP=0.83). Phenotypic similarity is not a clear distinguishing characteristic, especially in species with significant phenotypic divergence and small genetic differences. Most importantly, there is no evidence yet as to whether gene flow exists between *P. longliensis* and *P. zhijinensis*. Morphological analyses and karyotype data also support this conclusion. *P. longliensis* morphologically differs from *P. zhijinensis*. The posterior part of the upper branchial bone points upwards (vs. had outer gills) in *P. longliensis*, the fold of the tail fin behind the anus is red-orange and fades away at about 1/2 end of tail (vs. the fold of tail fin behind the anus being yellowy orange, and fades away at about 3/4 end of tail), there is a continuous earthen-yellow longitudinal stripe on each side of the abdomen (vs. lacking or intermittent), and *P. longliensis* has four pairs of submetacentric chromosomes (vs. two pairs in *P. zhijinensis*) (Figure 5: Li *et al.*, 2008a, 2008b; Sun *et al.*, 2015). Thus, both molecular and morphological analyses support *P. longliensis* and *P. zhijinensis* as valid species.

In this study, our data and analyses clearly demonstrate that the species diversity of the *P. caudopunctatus* species group has been underestimated. In addition to the five described species of the *P. caudopunctatus* species group, our results identified two distinct additional lineages that we consider as two candidates undescribed species (Figure 2 and Table 4). We found that two areas (DF and HS) consistently showed support for the existence of two putative species across the various species-delimitation methods used (Table 4). In our analysis, most species-delimitation methods supported the presence of two candidate species. The genetic distance values among the five lineages were variable, ranging from 0.61% (*P. longliensis* vs *P. maolanensis*) to 9.78% (*P. caudopunctatus* vs *P. longliensis*) (Table 3). Overall, the genetic distances between the two species in question and the other five genealogies are greater than the genetic distances between the accepted species within the *P. caudopunctatus* species group, and also are greater than the genetic distances between the other tailed amphibian species (1.1%, *Hynobius formosanus* vs *Hynobius arisanensis*; Pan *et al.*, 2019). Therefore, in this study, the species delimitation based on mitochondrial and nuclear gene sequences data revealed that there are multiple species in *P. caudopunctatus* species group. Of five species-delimitation methods used, four methods strongly supported the existence of seven species, *P. caudopunctatus*, *P. wulingensis*, *P. longliensis*, *P. maolanensis*, *P. zhijinensis*, *P. sp1* (HS),



**Figure 5** Dorsal view and ventral view of living specimens of the *P. caudopunctatus* species group. (A) *P. caudopunctatus*, GZNU 20190825001, adult male; (B) *P. wulingensis*, GZNU 20110719, adult male; (C) *P. zhijinensis*, GZNU 20190815002, adult male; (D) *P. longlisis*, GZNU 20190922002, adult male; (E) *P. sp2* (DF), GZNU 20180711001, adult male; (F) *P. sp1* (HS), GZNU 20180612001, adult male.

and *P. sp2* (DF). Underestimated species diversity indicates that the *P. caudopunctatus* species group evolved in response to complex species-forming mechanisms and a history of biological dispersal.

**Acknowledgments** This work was supported by the programs of the Strategic Priority Research Program B of

the Chinese Academy of Sciences (CAS) (No. XDB31000000), the National Natural Science Foundation of China (Grant No. 31460091), the National Animal Collection Resource Center, China (Grant No. 2005DKA21402), the Application of Amphibian Natural Antioxidant Peptides as Cosmetic Raw Material Antioxidants (QKZYD [2020]4002), the National Top Discipline Construction Project of Guizhou Province,



**Table 4** Number of lineages in the *P. caudopunctatus* species group inferred by multiple species delimitation methods.

Lineage	<i>n</i>	BF	GMYC single	GMYC multiple	bPTP	ABGD
A1	7	1	1	2	1	1
A2	9	1	1	1	1	1
B1	5	1	1	1	1	1
B2	2	1	1	1	1	1
B3	4	1	1	1	1	1
B4	4	1	1	1	1	1
B5	9	1	1	1	1	1
Total	40	7	7 (2–11)	7 (3–11)	6.55 (3–12)	7

Note: “*n*” represents the number of individuals; all bPTP are from Bayesian MCMC analyses. Confidence intervals for totals are in parentheses. ABGD results are based on the initial partitioning scheme with a maximum intraspecific diversity value of 0.00167 (K80 distances).

Geography in Guizhou Normal University (No. 85 2017 Qianjiao Keyan Fa). We thank Ms. Huan He for his help with sample collection. We thank Professor Paul A. Garber for editing assistance during the preparation of this manuscript. We thank LetPub (www.letpub.com) for its linguistic assistance during the preparation of this manuscript.

## References

- Bourret R. 1934. Notes herpétologiques sur l'Indochine française. VI. Sur diverses collections de serpents appartenants a l'Universite de Hanoi. VII. Une salamandre nouvelle vivant au Tonkin. Annexe au Bulletin Général de l'Instruction Publique. Hanoi, 1934: 83–84
- Baele G., Lemey P., Bedford T., Rambaut A., Suchard M. A., Alekseyenko A. V. 2012. Improving the accuracy of demographic and molecular clock model comparison while accommodating phylogenetic uncertainty. *Mol Biol Evol*, 29(9): 2157–2167
- Bergsten J., Nilsson A. N., Ronquist F., 2013. Bayesian tests of topology hypotheses with an example from diving beetles. *Syst Biol*, 62(5): 660–673
- Chang M. L. Y. 1935. Note préliminaire sur la classification des salamandres d'Asie orientale. *Bull Soc Zool (Paris)*, 60: 424–427
- Che J., Zhou W. W., Hu J. S., Yan F., Papenfuss T. J., Wake D. B., Zhang Y. P. 2010. Spiny frogs (Paini) illuminate the history of the Himalayan region and Southeast Asia. *Proc Nat Acad Sci USA*, 107(31): 13765–13770
- Chen J. M., Zhou W. W., Nikolay A., Poyarkov Jr., Stuart B. L., Brown R. M., Lathrop A., Wang Y. Y., Yuan Z. L., Jiang K., Hou M., Chen H. M., Suwannapoom C., Nguyen S. N., Duong T. V., Papenfuss T. J., Murphy R. W., Zhang Y. P., Che J. 2017. A novel multilocus phylogenetic estimation reveals unrecognized diversity in Asia toads, genus *Megophrys* sensu lato (Anura: Megophryidae). *Mol Phylogenet Evol*, 106: 28–43
- Drummond A. J., Suchard M. A., Xie D., Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. *Mol Biol Evol*, 29(8): 1969–1973
- Edgar R. C. 2004. MUSCLE: multiple sequence alignment with high accuracy and high throughput. *Nucleic Acids Res*, 32(5): 1792–1797
- Elsen P. R., Tingley M. W. 2015. Global mountain topography and the fate of montane species under climate change. *Nat Clim Change*, 5(8): 772–776.
- Fei L., Hu S. Q., Ye C. Y., Huang Y. Z. 2006. *Fauna Sinica, Amphibia Anura*, Vol. 1. Beijing, China: Science Press (In Chinese)
- Fei L., Ye C. Y., Jiang J. P. 2012. *Colored Atlas of Chinese Amphibians and their Distributions*. Chengdu, China: Sichuan Publishing House of Science and Technology (In Chinese)
- Fujisawa T., Barraclough T. G. 2013. Delimiting species using single-locus data and the generalized mixed Yule coalescent approach: a revised method and evaluation on simulated data sets. *Syst Biol*, 62(5): 707–724
- Fei L., Ye C. Y. 2016. *Amphibians of China*, Volume 1. Beijing, China: Science Press
- Favre A., Päckert M., Pauls S. U., Jähnig S. C., Uh D., Michalak I., Muellner-Riehl A. N. 2016. The role of the uplift of the Qinghai-Tibetan Plateau for the evolution of Tibetan biotas. *Biol Rev*, 90(1): 236–253
- Frost D. R. 2020. Amphibian species of the world: an online reference. version 6.0 [EB/OL] (2020-8-23). <http://research.amnh.org/herpetology/amphibia/index.html>
- Gray J. E. 1859. Descriptions of new species of salamanders from China and Siam. *Proc Zool Soc London*, 1859: 229–230
- Gu X. M., Chen R. R., Tian Y. Z., Li S., Ran J. C. 2012a. A new species of *Paramesotriton* (Caudata: Salamandridae) from Guizhou Province, China. *Zootaxa*, 3510(1): 41–52
- Gu X. M., Wang H., Chen R. R., Tian Y. Z., Li S. 2012b. The phylogenetic relationships of *Paramesotriton* (Caudata: Salamandridae) based on partial mitochondrial DNA gene sequences. *Zootaxa*, 3150(1): 59–68
- Grêt-Regamey A., Brunner S. H., Kienast F. 2012. Mountain ecosystem services: who cares? *Mt Res Dev*, 32(S1): S23–S34
- Grummer J. A., Bryson Jr R. W., Reeder T. W. 2014. Species delimitation using Bayes factors: simulations and application to the *Sceloporus scalaris* species group (Squamata: Phrynosomatidae). *Syst Biol*, 63(2): 119–133
- Guo P., Zhu F., Liu Q., Wang P., Che J., Nguyen T. Q. 2020. Out of the Hengduan Mountains: Molecular phylogeny and historical biogeography of the Asian water snake genus *Trimerodytes* (Squamata: Colubridae). *Mol Phy Evol*, 152: 106927
- Hu S. Q., Zhao E. M., Liu C. C. 1973. A survey of amphibians and reptiles in Kweichow province, including a herpetofaunal analysis. *Acta Zool Sinica*, 19(2): 160–163
- Huang Z. Y., Tang Z. Y., Tang Z. M. 1983. A new species of the genus *Trituroides* from Guangxi, China. *Acta Herpetol Sinica*, 2(2): 37–39

- Huson D. H., Bryant D. 2006. Application of phylogenetic networks in evolutionary studies. *Mol Biol Evol*, 23(2): 254–267
- Hoang D. T., Chernomor O., von Haeseler A., Minh B. Q., Le S. V. 2018. UFBoot2: Improving the ultrafast bootstrap approximation. *Mol Biol Evol*, 35(2): 518–522
- He K., Eliécer E. G., Heming N. M., Klaus P. K., Wan T., He S. W., Wei J., Liu S. Y., Jiang X. L. 2019. Cryptic phylogeographic history sheds light on the generation of species diversity in sky-island mountains. *J Biogeogr*, 46(10): 953–963
- Hu J., Huang Y., Jiang J., Guisan A. 2019. Genetic diversity in frogs linked to past and future climate change on the roof of the world. *J Anim Ecol*, 88(6): 953–963
- Jiang Z. G., Jiang J. P., Wang Y. Z., Zhang E., Zhang Y. Y., Li L. L., Xie F., Cai B., Cao L., Zheng G. M., Dong L., Zhang Z. W., Ding P., Luo Z. H., Ding C. Q., Ma Z. J., Tang S. H., Cao W. X., Li C. W., Hu H. J., Ma Y., Wu Y., Wang Y. X., Zhou K. Y., Liu S. Y., Chen Y. Y., Li J. T., Feng Z. J., Wang Y., Wang B., Li C., Song X. L., Cai L., Zang C. X., Zeng Y., Meng Z. B., Fang H. X., Ping X. G. 2016. Red List of China's Vertebrates. *Bio Sci*, 24(5): 500–551
- Jiang D. C., Klaus S., Zhang Y. P., Hillis D. M., Li J. T. 2019. Asymmetric biotic interchange across the bering land bridge between eurasia and north america. *Nati Sci Rev*, 6(4): 139–145
- Kass R. E., Raftery A. E. 1995. Bayes factors. *J Am Stat Assoc*, 90(430): 773–795.
- Körner C., Spehn E. M. 2002. Mountain Biodiversity: A Global Assessment. Boca Raton, FL: Parthenon Publishing Group
- Kumar S., Stecher G., Tamura K. 2016. MEGA7: molecular evolutionary genetics analysis version 7.0 for bigger datasets. *Mol Biol Evol*, 33(7): 1870–1874
- Li S., Tian Y. Z., Gu X. M. 2008a. A new species of the genus *Paramesotriton* (Caudata, Salamandridae). *Acta Zootaxonomica Sin*, 33(2): 410–413 (In Chinese)
- Li S., Tian Y. Z., Gu X. M., Xiong R. C. 2008b. A new species of *Paramesotriton longliensis* (Caudata: Salamandridae). *Zoo Res*, 29(3): 313–317 (In Chinese)
- Lanfear R., Calcott B., Ho S. Y. W., Guindon S. 2012. PartitionFinder: combined selection of partitioning schemes and substitution models for phylogenetic analyses. *Mol Biol Evol*, 29(6): 1695–1701
- Myers G. S., Leviton A. E. 1962. The Hong Kong newt described as a new species. *Occa Pap Div Syst Biol*, 10: 1–4
- Monaghan M. T., Wild R., Elliot M., Fujisawa T., Balke M., Inward D. J. G., Lees D. C., Ranaivosolo R., Eggleton P., Barraclough T. G. 2009. Accelerated species inventory on Madagascar using coalescent-based models of species delineation. *Syst Biol*, 58(3): 298–311
- McCain C. M., Colwell R. K. 2011. Assessing the threat to montane biodiversity from discordant shifts in temperature and precipitation in a changing climate. *Ecol Lett*, 14(12): 1236–1245
- Nguyen L. T., Schmidt H. A., Von H. A., Minh B. Q. 2014. IQ-TREE: a fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. *Mol Biol Evol*, 32(1): 268–274
- Puillandre N., Lambert A., Brouillet S., Achaz G. 2012. ABGD, Automatic Barcode Gap Discovery for primary species delimitation. *Mol Ecol*, 21(8): 1864–1877
- Pan T., Sun Z., Lai X., Orozco-terwengel P., Yan P., Wu G. Y., Wang H., Zhu W. Q., Wu X. B., Zhang B. W. 2019. Hidden species diversity in Pachyhydnobius: A multiple approaches species delimitation with mitogenomes. *Mol Phyl Evol*, 137: 138–145
- Rambaut A., Drummond A. 2007. Tracer v1.4. Available from <http://beast.bio.ed.ac.uk/Tracer>
- Ronquist F., Teslenko M., Mark P. V. D., Ayres D., Darling A., Hohna S., Larget B., Liu L., Suchard M. A., Huelsenbeck J. P. 2012. MrBayes 3.2: efficient Bayesian phylogenetic inference and model choice across a large model space. *Syst Biol*, 61(3): 539–542
- Sambrook J., Fritsch E. F., Maniatis T. 1989. Molecular cloning. New York, USA: Cold Spring Harbor Laboratory Press
- Sun H. L. 1997. Research of the formation, environment change on Qinghai-Xizang (Tibetan) Plateau. Changsha, China: Hunan Science and Technology Press (In Chinese)
- Shi Y. F., Cui Z. J., Su Z. 2006. The Quaternary glaciations and environmental variations in China. Shijiazhuang, China: Hebei Science and Technology Press (In Chinese)
- Stuart B. L., Phimmachak S., Sivongxay N., Robichaud W. G. 2010. A new species in the *Tylosotriton asperimus* group (Caudata: Salamandridae) from central Laos. *Zootaxa*, 2650(1): 19–32
- Sun A. Q., Tian Y. Z., Li S., Xiong R. C., Chen H. 2015. Comparative studies on karyotypes of *Paramesotriton longliensis* and *P. zhijinensis*. *Sichuan J Zool*, 34(1): 81–83 (In Chinese)
- Unterstein W. 1930. Beiträge zur Lurch-und Kriechtierfauna Kwangsi's. 2. Schwanzlurche. *Sit Gesellsch Naturforsch Freunde*, 1930(8–10): 313–315
- Vieites D. R., Min M. S., Wake D. B. 2007. Rapid diversification and dispersal during periods of global warming by plethodontid salamanders. *Proc Nat Acad Sci USA*, 104(50): 19903–19907
- Wen Y. T. 1989. A new species of the genus *Paramesotriton* (Amphibia: Caudata) from Guangxi and a comparison with *P. guangxiensis*. *Chin Herpetol Res*, 2(2): 15–20
- Wu Y. K., Rovito S. M., Papenfuss T. J., Hanken J. 2009. A new species of the genus *Paramesotriton* (Caudata: Salamandridae) from Guangxi Zhuang Autonomous Region, southern China. *Zootaxa*, 2060(1): 59–68
- Wu Y. K., Jiang K., Hanken J. 2010. A new species of newt of the genus *Paramesotriton* (Salamandridae) from southwestern Guangdong, China, with a new northern record of *P. longliensis* from western Hubei. *Zootaxa*, 2494(1): 45–58
- Wang C., Tian Y. Z., Gu X. M. 2013. A new species of the genus *Paramesotriton* (Caudata, Salamandridae). *Acta Zoot Sin*, 38(2): 388–397 (In Chinese)
- Wang B., Nishikawa K., Matsui M., Nguyen T. Q., Xie F., Li C., Khatiwada J. R., Zhang B. W., Gong D. J., Mo Y. M., Wei G., Chen X. H., Shen Y. H., Yang D. D., Xiong R. C., Jiang J. P. 2018. Phylogenetic surveys on the newt genus *Tylosotriton sensu lato* (Salamandridae, Caudata) reveal cryptic diversity and novel diversification promoted by historical climatic shifts. *PeerJ*, 6: e4384
- Wu Y. H., Yan F., Stuart B. L., Prendini E., Suwannapoom C., Dahn H. A., Zhang B. L., Cai H. X., Xu Y. B., Jiang K., Chen H. M., Alan R. L., Lemmon Emily A. R., Lemmon E. M., Raxworthy C. J., Orlov N. L., Murphy R. W., Che J. 2020. A combined approach of mitochondrial DNA and anchored nuclear phylogenomics sheds light on unrecognized diversity, phylogeny, and historical biogeography of the torrent frogs, genus *Amolops* (Anura: Ranidae). *Mol Phyl Evol*, 148: 106789
- Xie W., Lewis P. O., Fan Y., Kuo L., Chen M. H. 2011. Improving marginal likelihood estimation for Bayesian phylogenetic model selection. *Syst Biol*, 60(2): 150–160
- Yan F., Zhou W. W., Zhao H. T., Yuan Z. Y., Wang Y. Y., Jiang K., Jin J. Q., Murphy R. W., Che J., Zhang Y. P. 2013. Geological events play a larger role than Pleistocene climatic fluctuations in driving the genetic structure of *Quasipaa boulengeri* (Anura: Dicroglossidae). *Mol Ecol*, 22(4):



- 1120–1133
- Yu Y., Harris A. J., He X. J. 2014. RASP (Reconstruct Ancestral State in Phylogenies): a tool for historical biogeography. *Mol Phylogenet Evol*, 87: 46–49
- Yuan Z. Y., Zhao H. P., Jiang K., Hou M., He L. Z., Murphy R. W., Che J. 2014. Phylogenetic relationships of the genus *Paramesotriton* (Caudata: Salamandridae) with the description of a new species from Qixiling Nature Reserve, Jiangxi, southeastern China and a key to the species. *Asian Herpetol Res*, 5(2): 67–79
- Yuan Z. Y., Wu Y. K., Zhou J. J., Che J. 2016a. A new species of the genus *Paramesotriton* (Caudata: Salamandridae) from Fujian, southeastern China. *Zootaxa*, 4205(6): 549–563
- Yuan Z. Y., Zhou W. W., Chen X., Poyarkov Jr. N. A., Chen H., Jang-Liaw N., Chou W., Matzke N. J., Iizuka K., Min M., Kuzmin S. L., Zhang Y., Cannatella D. C., Hillis D. M., Che J. 2016b. Spatiotemporal diversification of the true frogs (Genus *Rana*): a historical framework for a widely studied group of model organisms. *Syst Biol*, 65(2): 824–842
- Yao M. C., Wei M. C., Nie H. Y. 2018. Geographic distribution pattern and dispersal route of Urodela in China. *Chinese J Zool*, 53(1): 1–16 (In Chinese)
- Yan F., Lü J. C., Zhang B. L., Yuan Z. Y., Zhao H. P., Huang S., Wei G., Mi X., Zou D. H., Xu W., Chen S., Wang J., Xie F., Wu M. Y., Xiao H. B., Liang Z. Q., Jin J. Q., Wu S. F., Xu C. S., Tapley B., Turvey S. T., Papenfuss T. J., Cunningham A. A., Murph R. W., Zhang Y. P., Che J. 2018. The Chinese giant salamander exemplifies the hidden extinction of cryptic species. *Curr Biol*, 28(10): 590–592
- Yuan Z. Y., Zhang B. L., Raxworthy C. J., Weisrock D. W., Hime P. M., Jin J. Q., Lemmon E. M., Lemmon A. R., Holland S. D., Kortyna M. L., Zhou W. W., Peng M. S., Che J., Prendini E. 2018. Natatanuran frogs used the Indian Plate to step-stone disperse and radiate across the Indian Ocean. *Nat Sci Rev*, 6(1): 10–14
- Zhou Q. Y., Chen P. Y. 1993. Lithofacies change and palaeogeographical evolution during Late Cenozoic in Guizhou and its vicinity. *Geol Guizhou*, 10(3): 201–207 (In Chinese)
- Zhang P., Papenfuss T. J., Wake M. H., Qu L. H., Wake D. B. 2008. Phylogeny and biogeography of the family Salamandridae (Amphibia: Caudata) inferred from complete mitochondrial genomes. *Mol Phylogenet Evol*, 49(2): 586–597
- Zhang J., Kapli P., Pavlidis P., Stamatakis A. 2013. A general species delimitation method with applications to phylogenetic placements. *Bioinformatics*, 29(22): 2869–2876
- Zhou Y., Wang S., Zhu H., Li P., Yang B., Ma J. 2017. Phylogeny and biogeography of South Chinese brown frogs (Ranidae, Anura). *PLoS One*, 12(4): e0175113

Handling Editor: Heling Zhao

**How to cite this article:**

**Luo T., Wen H. M., Gao K., Zhou J., Zhou J.** Phylogeography and Cryptic Species Diversity of *Paramesotriton caudopunctatus* Species Group (Salamandridae: *Paramesotriton*) in Guizhou, China. *Asian Herpetol Res*, 2021, 12(2): 188–200. DOI: 10.16373/j.cnki.ahr.200025